

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Applicant's or agent's file reference 40971		Date of mailing (day/month/year) 20 -08- 2004	
		FOR FURTHER ACTION See paragraph 2 below	
International application No. PCT/FI2004/000228	International filing date (day/month/year) 14.04.2004	Priority date (day/month/year) 14.04.2003	
International Patent Classification (IPC) or both national classification and IPC C12N 15/90, C12N 15/79			
Applicant Finnzymes Oy et al			

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further opinions, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - ☐ This opinion has been established on the basis of a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing
 - ☒ contained in the international application as filed.
 - ☒ filed together with the international application in computer readable form.
 - ☐ furnished subsequently to this Authority for the purposes of search.
3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

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Box No. II Priority

1. ☒ The following document has not yet been furnished:

☒ copy of the earlier application whose priority has been claimed (Rules 43*bis*.1 and 66.7(a)).

☐ translation of the earlier application whose priority has been claimed (Rules 43*bis*.1 and 66.7(b)).

Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

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Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	2-3, 6, 8, 10-11	YES
	Claims	1, 4-5, 7, 9	NO
Inventive step (IS)	Claims		YES
	Claims	1-11	NO
Industrial applicability (IA)	Claims	1-11	YES
	Claims		NO

2. Citations and explanations:

The following documents are considered relevant:

D1) US2002/0132350 A1

D2) Lamberg et al "Efficient insertion mutagenesis strategy for bacterial genomes involving electroporation of in vitro-assembled DNA transposition complexes of bacteriophage mu", Appl. Environ. Microbiol. 2002 Feb;68(2):705-12

D3) Goryshin et al: "Insertional transposon mutagenesis by electroporation of released Tn5 transposition complexes", Nat Biotechnol. 2000 Jan;18(1):97-100

D4) Shi et al: "Efficient transposition of preformed synaptic Tn5 complexes in Trypanosoma brucei", Mol Biochem Parasitol. 2002 Apr 30;121(1):141-4

D5) US6294385 B1

D1 shows a method for targeted genetic manipulation of e.g. maize and soybean cells. Active cleaved donor complex (CDC) comprising Mu sequences and MuA is transformed into maize and soybean cells by microprojectile bombardment. Reporter genes and nuclear localisation sequences can be included. See Figure 4, [0038], [0041] lines 1-3, [0042], [0046]-[0047] line 9, [0058], [0100] lines 1-14, [0156], p. 24-25 examples 6-7 and [0188].

D2 shows the assembly of integration-proficient Mu transposition complexes that, after introduction into bacterial cells by electroporation, execute transposon integration into bacterial chromosomes. See abstract, p. 705 right col. last paragraph- p. 707 left col. paragraph 1

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: BOX V

and p. 711 left col. paragraph 3-4.

D3 shows that premade Tn5 synaptic complexes can transpose in the yeast *Saccharomyces cerevisiae*. This is shown by electroporation of a Tn5 transposome into *S. cerevisiae*. See abstract and p. 99 left col. paragraphs 5 and 7.

D4 shows that in vitro preformed Tn5 synaptic complexes can insert into the genome of *T. brucei*. See abstract, p. 141 left col. paragraph 2- right col. paragraph 2, p. 142 figure 1B and p. 143 left col. paragraph 2.

D5 shows synaptic Tn5 complexes formed in vitro delivered into target cells. Libraries of cells are contemplated. See col. 2 lines 50-59, col. 2 line 66-col. 3 line 4, col. 3 line 63-col. 4 line 4 and col. 8 lines 32-40.

The present application relates to the introduction of in vitro-assembled DNA transposition complexes into eukaryotic cells. One benefit is that there is no need to generate an expression system of the transposition machinery for the organism of interest.

Given the method shown in D1, the invention according to claims 1, 4-5, 7, 9 lacks novelty. In D1, the CDC inserted into the cell has the intact MuA tetrameric core attached.

The additional aspects of claims 2-3, 6, 8 and 10-11 are considered as particular detailed executions obvious to a skilled person. Therefore, claims 2-3, 6, 8 and 10-11 are considered to lack an inventive step given what is shown in D1.

Of special interest are also documents D2-D5:
In D2 it is stated that the strategy disclosed therein also could be "applicable to gram-positive bacteria and perhaps to some eukaryotic organisms (such as yeast) as well". Consequently, D2 leads the skilled person to the invention according to claim 1.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: BOX V

In D3, in vitro assembled Tn5 synaptic complexes are shown to transpose in the yeast *Saccharomyces cerevisiae*. The difference between the invention according to claim 1 and D3 is that Mu transposition complexes are used instead of Tn5 complexes. However, the skilled person knows that a common DNA transposition mechanism is shared among a variety of mobile elements and would thus consider Mu as an alternative to Tn5. The same argumentation can be made starting with D4, which show transposition of preformed synaptic Tn5 complexes in the eukaryote *Trypanosoma brucei*. Consequently, the invention according to claim 1 is considered not to involve an inventive step given what is shown in D3 or D4.

In D5, in vitro assembled Tn5 synaptic complexes are delivered into target cells. In the example, *E. coli* is transformed, but it is stated that no scientific impediment is known to exist that would prevent use of the method in e.g. plant and animal cells. Since the skilled person is aware that a common DNA transposition mechanism is shared among a variety of mobile elements, she would consider Mu as an alternative to Tn5. Consequently, in analogy with what is said above about D3, the invention according to claim 1 is considered obvious to the skilled person in view of D5.

The features of claims 2-11 represent modifications obvious to the skilled person.

Consequently, the invention according to claims 1-11 is considered not to involve an inventive step given what is disclosed in either one of documents D2-D5.

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Box No. VI Certain documents cited

1. Certain published documents (Rules 43bis.1 and 70.10)

<u>Application No. Patent No.</u>	<u>Publication date (day/month/year)</u>	<u>Filing date (day/month/year)</u>	<u>Priority date (valid claim) (day/month/year)</u>
US2003/0143740	31/07/2003	15/10/2002	

2. Non-written disclosures (Rules 43bis.1 and 70.9)

<u>Kind of non-written disclosure</u>	<u>Date of non-written disclosure (day/month/year)</u>	<u>Date of written disclosure referring to non-written disclosure (day/month/year)</u>
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